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PCT/SE00/01380

## PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

ASTRAZENECA AB  
 AstraZeneca R&D Headquarters  
 Global Intellectual Property  
 S-151 85 Sodertalje  
 SUÈDE

Date of mailing (day/month/year) 04 février 2002 (04.02.02)	
Applicant's or agent's file reference RE: 100587	<b>IMPORTANT NOTIFICATION</b>
International application No. PCT/SE00/01380	International filing date (day/month/year) 29 juin 2000 (29.06.00)

1. The following indications appeared on record concerning:				
<input checked="" type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input type="checkbox"/> the agent	<input type="checkbox"/> the common representative	
Name and Address  KAROLINSKA INNOVATIONS AB S-171 77 Stockholm Sweden	State of Nationality		State of Residence	
	SE		SE	
	Telephone No.			
	Facsimile No.			
Teleprinter No.				

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:				
<input checked="" type="checkbox"/> the person	<input type="checkbox"/> the name	<input type="checkbox"/> the address	<input type="checkbox"/> the nationality	<input type="checkbox"/> the residence
Name and Address  ASTRAZENECA AB S-151 85 Sodertalje Sweden	State of Nationality		State of Residence	
	SE		SE	
	Telephone No.			
	Facsimile No.			
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3. Further observations, if necessary:				

4. A copy of this notification has been sent to:				
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/>	the designated Offices concerned		
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The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No.: (41-22) 740.14.35	Authorized officer  Beatrix LARGO  Telephone No.: (41-22) 338.83.38
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/01380

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC7: C12N 15/00, C01K 14/705, A61K 39/395, A61K 67/027**  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**IPC7: C12N, C17K, A61K, A01K**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**SE,DK,FI,NO classes as above**

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5783680 A (H.G. BRUNNER ET AL), 21 July 1998 (21.07.98), see especially column 1, line 59 - column 2, line 59  --	1-16,18-21, 23-31
Y	SCIENCE, Volume 276, April 1997, Rolf H. Zetterström et al, "Dopamine Neuron Agenesis in Nurr1-Deficient Mice", page 248 - page 580, see especially abstract  --	1-16,18-21, 23-31
A	Proc. Natl. Acad. Sci. USA, Volume 95, March 1998, ODILA SAUCEDO-CARDENAS et al, "Nurr1 essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons", page 4013 - page 4018, see entire document  --	1-16,18-21, 23-31

Further documents are listed in the continuation of Box C.

See patent family annex.

- \* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

23 October 2000

Date of mailing of the international search report

26-10-2000

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Henrik Nilsson/GH  
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# INTERNATIONAL SEARCH REPORT

International application No.  
SE00/01380

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: **17, 22**  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
**see extra sheet \***
2.  Claims Nos.: **29-31**  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
**see extra sheet \*\***
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**International application No.  
SE00/01380

\*  
Present claims 17 and 22 relate to substances defined by reference to a desirable characteristic or property, namely that they can be obtained by the methods disclosed in claims 16 and 21. The claims cover all substances having this characteristic or property, whereas the application does not appear to provide support within the meaning of Article 6 or disclosure within the meaning of Article 5 PCT for any substance. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). Defining a substance by reference to the method by which it can be obtained is in most cases not acceptable, due to the fact that the scope of the claim can not be adequately defined. This lack of clarity in the present case is such as to render a meaningful search over the claimed scope impossible. Consequently, claims 17 and 22 have not been searched.

\*\*

Claims 29-31 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

03/10/00

International application No.

PCT/SE 00/01380

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5783680 A	21/07/98	NONE	

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/01380

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- \* Special categories of cited documents:
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

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Date of mailing of the international search report

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Authorized officer  
 Henrik Nilsson/GH  
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**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

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1.  Claims Nos.: 17, 22  
because they relate to subject matter not required to be searched by this Authority, namely:

**see extra sheet \***

2.  Claims Nos.: 29-31  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

**see extra sheet \*\***

3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

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1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
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**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest.  
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\*

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\*\*

Claims 29-31 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

03/10/00

International application No.

PCT/SE 00/01380

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5783680 A	21/07/98	NONE	

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
4 January 2001 (04.01.2001)

PCT

(10) International Publication Number  
**WO 01/00807 A1**(51) International Patent Classification<sup>7</sup>: C12N 15/00, (74) Agent: KILANDER, Annika; Göteborgs Patentbyrå Dahls AB, P.O. Box 606, S-182 16 Danderyd (SE).

(21) International Application Number: PCT/SE00/01380

(22) International Filing Date: 29 June 2000 (29.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
9902489-5 30 June 1999 (30.06.1999) SE(71) Applicant (for all designated States except US):  
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(75) Inventors/Applicants (for US only): BUERVENICH, Silvia [DE/SE]; Berga Backe 6, S-182 53 Danderyd (SE). OLSON, Lars [SE/SE]; Ankarvägen 1, S-181 43 Lidingö (SE). ANVRET, Maria [SE/SE]; Statyvägen 4, S-175 47 Järfälla (SE). CARMINE, Andrea [SE/SE]; Byggmästarvägen 6, S-168 32 Bromma (SE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- With international search report.
- With amended claims.

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**WO 01/00807 A1**

(54) Title: MUTATED Nurr1 GENE

(57) Abstract: The present invention relates to an isolated Nurr1 gene including one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del), or a functional fragment or variant thereof, as well as to proteins or peptides encoded thereof. Further, the invention also relates to cell cultures and transgenic animals comprising a mutated gene or a gene fragment as models for the study of psychotic disorders, such as schizophrenia and/or manic depressive disorder, as well as for the identification of effective therapies and drugs for the treatment of said disorders. In an additional aspect the invention relates to novel drugs developed by use of one or more of the mutations according to the invention for the treatment and/or prevention of psychotic disorders. Finally, the invention relates to methods of diagnosis wherein the mutations according to the invention are identified as well as to kits for performing such methods.

MUTATED Nurr1 GENETechnical field

The present invention relates to novel mutations identified for the first time in the  
5 Nurr1 gene.

Background

Clinical conditions associated with defects of specific genes may be inherited or have alternatively been caused by a later developed mutation in the afflicted individual.  
10 It is assumed that many neurological and psychiatric conditions have hereditary components, and indeed, schizophrenia, bipolar affective disorder and Parkinson's disease have in common that affected relatives constitute the largest epidemiological risk factor. However, the mode of inheritance of susceptibility is complex and has not yet been elucidated. A further common observation in said three different diseases is etiologic and/or therapeutic involvement of dopaminergic neurotransmission. Thus, cells of the mesostriatal midbrain dopamine (DA) neuron system degenerate in Parkinson's disease (Hornykiewicz, O. Biochemical aspects of Parkinson's disease. *Neurology* **51**, S2-S9 (1998)), while the mesolimbic DA axis is the target of several antipsychotic drugs (Gerner, R.H., Post, R.M. & Bunney, W.E.J. A  
15 dopaminergic mechanism in mania, *Am J. Psychiatry* **133**, 1177-1180 (1976); and Creese, I., Burt, D.R. & Snyder, S.H. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* **192**, 481-483 (1976)). Recently, a number of studies have pointed out the importance of retinoids and retinoid-related genes for DA cells. Midbrain DA neurons express receptors for  
20 retinoic acid-mediated transcription, and mice lacking the retinoid-related receptor Nurr1 fail to develop mesencephalic DA neurons (Zetterström, R.H. *et al.* Dopamine neuron aenesis in Nurr1-deficient mice. *Science* **276**, 248-250 (1997)).

The immediate early gene Nurr1 (also called *NOT* in humans, classified as NR4A2  
30 according to the most recent nomenclature [Nuclear Receptors Nomenclature

Committee, 1999]) codes for a nuclear orphan receptor of the NGFI-B family of transcription factors (Gerner et al., *supra*; Hazel, T.G., Nathans, D. & Lau, L.F. A gene inducible by serum growth factors encodes a member of the steroid and thyroid hormone receptor superfamily. *Proc Natl Acad. Sci. USA* **85**, 8444-8448  
5 (1988); and Mages, H.W., Rilke, O., Bravo, R., Senger, G, G. & Kroczeck, R.A. NOT, a human immediate-early response gene closely related to the steroid/thyroid hormone receptor NAK1/TR3. *Mol. Endocrinol.* **8**, 1583-1591 (1994)). The major splice variant is a 598 amino acid protein with ligand binding, DNA binding and N-terminal domains. Recently, further alternatively spliced mRNA variants encoding  
10 the entire N-terminal and DNA binding domains but shorter C-terminal domains have been isolated (Ichinose, H. Et al. Molecular cloning of the human Nurr1 gene: characterization of the human gene and cDNAs. *Gene* **230**, 233-239 (1999); Torii, T., Kawarai, T., Nakamura, S. & Kawakami, H. Organization of the human orphan nuclear receptor Nurr1 gene. *Gene* **230**, 225-232 (1999); and Ohkura, N., Hosono,  
15 T., Maruyama, K., Tsukada, T. & Yamaguchi, K. An isoform of Nurr1 functions as a negative inhibitor of the NGFI-B family signaling. *Biochim. Biophys. Acta* **1444**, 69-79 (1999)). While no activating ligand for Nurr1 has been identified to date, it has been shown that Nurr1 can be both constitutively active as a transcription factor and dimerize with RXR, a receptor involved in mediating retinoic-acid induced  
20 transcription (Perlmann, T. & Jansson, L. A novel pathway for vitamin A signaling mediated by RXR heterodimerization with NGFI-B and Nurr1. *Genes Dev.* **9**, 769-782 (1995)). These studies together with other observations have made Nurr1 a suggestive candidate gene for diseases with an involvement of the dopamine neuron system, such as schizophrenia, manic depressive illness and Parkinson's disease. However, up to date, no evidence has been provided showing any such involvement, and thus, no effective cures have yet been developed based on such genetic knowledge. As about 1% of the GDP of an industrial country each year is spent on patients suffering from diseases such as schizophrenia and bipolar affective disorder, a clear understanding of the genetic background could hopefully lead to effective treatment schemes and diagnoses. Accordingly, within this field, there is a strong need  
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based both on human and economical factors of gaining a more thorough understanding.

Previously, certain genes associated with specific, mental conditions have been disclosed. For example, US 5 783 680 discloses monoxidase genes and proteins associated with abnormal behavior. More specifically, the conditions defined therein are behavioural disorders, such as impulsive aggression, and include borderline mental retardation and aggressive outbursts, often in response to anger, fear or frustration. Such aggressive behaviour was noted to vary markedly in severity. Other types of impulsive behaviour that occurred in individual cases included arson, attempted rape, and exhibitionism. Thus, as is obvious to one of skill in this field, there are clear differences between the conditions which US 5 783 680 relates to and psychiatric disease, such as schizophrenia. This is evident from the fact, that none of the symptoms cited in said US 5 783 680 are found in the DSM-IV diagnostic criteria for schizophrenia or manic depression, nor are the minimal features required for schizophrenia or manic depression present in the family mentioned in US 5 783 680. (For the DSM-IV criteria, see Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Copyright 1994 American Psychiatric Association.) Thus, US 5 783 680 does not relate to mutations that can cause psychiatric disturbances.

Finally, Zetterström et al (Science, vol. 276, 11 April, 1997: "Dopamine Neuron Agenesis in Nurr1-Deficient Mice") have shown that mice that lack Nurr1 fail to generate midbrain dopaminergic neurons and suggest Nurr1 ligands as useful for the treatment of Parkinson's disease.

#### Summary of the invention

For the first time, the present invention provides three mutations of the human Nurr1 gene as defined in the appended claim 1 and further shows the relation thereof with a previously unreported subgroup of ca. 1% of the conditions schizophrenia

and bipolar affective disorder. The novel mutations according to the invention are herein denoted M1, M2 and M3.

Brief description of the drawings

- 5      Figure 1 is an overview of the genomic structure of the human Nurr1 gene, distribution of PCR fragments 0-7 and localization of mutations M1-M3 according to the invention in exon 3.
- Figure 2 shows autoradiographs of the three heterozygous mutations M1-M3 according to the invention.
- 10     Figure 3 shows in table 1 the primers used to amplify the PCR fragments 0 to 7.
- Figure 4 provides by table 2 clinical descriptions of the three heterozygous carriers of mutations M1 to M3 according to the invention.

Definitions

- 15     In the present application, the term Nurr1 is intended to encompass both mammalian Nurr1 genes, present for example in humans or rodents, and Nurr1 genes of non-mammalian species, such as zebrafish or lamprey. Further, all genes, gene fragments or cDNA species identical to Nurr1 and its cDNA, but published under other names (for example NOT (in humans), RNR1 (in rats) and HZF-3 (in rats)) are also encompassed by the term Nurr1 as used herein. Thus, any mode of writing such as Nurr1, *Nurr1*, NURR1 and *NURR1* as used herein is to be understood to encompass all of these aspects.
- 20

- In the present application, the term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides. When reference is made to the gene, it is to be understood that both DNA and cDNA are encompassed.

The term "variant" as used herein means any functional equivalents of the Nurr1 gene including naturally occurring genes present in other species, and mutants, whether naturally occurring or engineered.

- 5 In the present specification, the term "highly stringent conditions" means hybridisation to filter-bound DNA in 0.5M NaHPO<sub>4</sub>, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65<sup>0</sup>C and washing in 0.1x SSC/0.1% SDS at 68<sup>0</sup>C (Ausubel F. M. *et al.*, eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3). In instances  
10 wherein the nucleic acid molecules are deoxyoligonucleotides ("oligos"), highly stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37<sup>0</sup>C (for 14-base oligos), 48<sup>0</sup>C (for 17-base oligos), 55<sup>0</sup>C (for 20-base oligos), and 60<sup>0</sup>C (for 23-base oligos). Low stringency conditions are well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions see, for example, Sambrook *et al.*, 1989, *Molecular Cloning, A Laboratory Manual*, Cold Springs Harbor Press, N.Y.; and Ausubel *et al.*, 1989, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley, Interscience, N. Y.  
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#### Detailed description of the invention

Thus, in a first aspect, the present invention relates to an isolated Nurr1 gene including one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122 (Y122del), or a functional fragment or variant thereof. The fragments encompassed by the present invention may comprise one or more of the present mutations surrounded by wild type sequence or adjacent to wild type sequence on one side thereof. In this context, reference is made to Figure 1 showing an overview of the genomic structure of Nurr1 and the mutations according to the invention. The human Nurr1 gene is comprised of 8 kb as 8 exons and 7 introns located on chromosome 2q22-23, see Ichino-

se et al., *Gene* 230 (1999) 133-239, which gene has been deposited in the DDBJ/EMBL/GenBank with accession number AB017586. (For the entire sequence of mouse Nurr1, see Castillo et al., *Genomics* 41, 250-257 (1997), Fig 1, accession no. U86783.) In one embodiment, a fragment according to the invention comprises  
5 all the exons of the Nurr1 gene, i.e all of the cDNA sequence thereof. More specifically, as the herein defined novel mutations are present on exon 3, in one embodiment, a fragment according to the invention comprises at least exon 3 of Nurr1. Specific embodiments of the first aspect are fragments comprising the mutation Met97Val, wherein a G in the first position of codon no 97 has substituted an A in  
10 said position, the mutation His103Arg, wherein a G in the first position of codon no 103 has substituted an A in said position, or the mutation Tyr121del or Tyr122del, wherein a tyrosine has been deleted at position 121 or 122. As easily appreciated by the skilled in this field, and as further discussed in relation to figure 2, the last mentioned deletions are impossible to differentiate from each other, and therefore they  
15 will be denoted Tyr122 herein. The present fragments may include any suitable length of the Nurr1 gene depending on the intended use. Further, in the present context, it is noted that the present invention relates to mutated Nurr1 genes of human and mouse origin, irrespective of whether it is written in capital letters or not. Various advantageous uses of the present genes and fragments will be discussed in  
20 further detail below.

Accordingly, by disclosing the novel mutations defined above, the present invention shows for the first time a linkage between the Nurr1 gene and neuro-psychiatric disorders, which linkage has previously been sought for without any success, see e.g.  
25 Ichinose et al., *supra*. Previously, when such a possible link was discussed, a general connection to neuro-psychiatric conditions was suggested but never substantiated. However, it has never been proposed or foreseen that such a link would exist only to a specific selection of said conditions. Accordingly, the evidence presented herein that mutations in the Nurr1 gene are linked to schizophrenia and manic depressive illness, but not to Parkinson's disease, is highly surprising and will enable  
30

an essential step forward in studies aimed at developing new drugs as well as new diagnostic tools within this field. Furthermore, the invention is of importance for the diagnostic field of schizophrenia and bipolar affective disorder because the finding of Nurr1 mutations in a subgroup of patients will help to better characterize the ill-defined and inhomogenous group of patients suffering from these conditions. Thus, those patients suffering from schizophrenia or bipolar affective disorder because of mutated Nurr1 can now be identified and treated differently and more specifically according to their specific genetic defect, which has been revealed for the first time in the present invention.

10

Thus, even though Zetterström et al (Science, 1997, *supra*) suggested that putative Nurr1 ligands could be useful for the treatment of Parkinson's disease and other disorders of midbrain dopamine circuitry, they could not show any link between Nurr1 and the herein discussed conditions of schizophrenia and manic depression.

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The present invention discloses for the first time three specific mutations which as shown below in the experimental section are clearly linked to a subgroup of patients suffering from said conditions. Although the three disclosed mutations cannot explain the condition in all schizophrenia or manic depressed patients, their absence from investigated control material strengthens their role in the patients where they have been identified. Since an effect of each one of the mutations on Nurr1 activity has been shown, and all three mutations show very similar effects, reliable evidence is provided that the present mutations contribute to the phenotype observed in those patients in which mutations were found.

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To further stress the practical usefulness of the present invention, it is noted that a person skilled in the art is well aware of the multifactorial etiology of complex diseases, such as schizophrenia and manic depression. It is therefore unlikely that one would find mutations in a single gene in a large portion of patients. Given a worldwide prevalence of both diseases of about 1% each, explaining 1% of these diseases can help an enormous number of people.

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Actually, after above discussed description of mutations in MAOA in patients with impulsive aggression (US 5 783 680), another study (Am J Med Genet 1999 Feb 5;88(1):25-8) was carried out searching for MAOA mutations in patients with similar diagnoses (and also healthy individuals and Parkinson patients who served as control material), without finding any mutations. Thus, similarly to the novel mutations according to the present invention, MAOA mutations are very rare causes of certain conditions, which in their case are behavioural.

- 10 Another aspect of the present invention is a nucleic acid capable of specifically hybridising to a gene or a fragment as defined above. The preparation of DNA is easily accomplished by the skilled in this field by any suitable method, such as cloning and restriction of appropriate sequences by any *in vitro* or *in vivo* method, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (SSR) or cell based cloning and amplification, wherein the cell may be any suitable cell, such as a bacterium, a cultured cell line etc. A wide variety of cloning and amplification methods are well known to the skilled in this field, see e.g. Sambrook *et al.*, (1989); *Molecular Cloning: A Laboratory Manual*, 2nd Ed, Vol. 1-3).
- 15 Further, they may be prepared by direct chemical synthesis by methods such as the phosphotriester method (Narang *et al.*, *Meth. Enzymol.* **68**, 90-99 (1979); the phosphodiester method (Brown *et al.*, *Meth. Enzymol.* **68**, 109-151 (1979); the diethylphosphoramidite method (Beaucage *et al.*, *Tetra. Lett.*, **22**, 1859-1862 (1981); and the solid support method (US patent no. 4 458 066).
- 20

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The present fragments may e.g. be used as primers, e.g. for PCR, which for example may be present in a kit, as probes etc. Further, as discussed below, they may be used to generate immunogenic polypeptides or fusion proteins for use in generating specific antibodies which recognise the mutant epitope. For use as primers, the frag-

ments are preferably of a length of at least about 10, such as at least about 15-20, bases, which however depends on the method used.

A second aspect of the invention is a vector comprising a nucleic acid according to  
5 the invention as well as to cells harbouring such a nucleic acid useful for the pro-  
duction of the present protein or peptide. Such cells may be procaryotic or eucaryo-  
tic and are described in more detail below in relation to cell culture model systems.

A third aspect of the invention is a protein or a peptide encoded by a gene or a gene  
10 fragment according to the invention. Accordingly, in specific embodiments, the pre-  
sent protein or polypeptide may include a Val residue in the position corresponding  
to amino acid no. 97 of the wild type Nurr1 protein; an Arg residue in the position  
corresponding to amino acid no. 103 of the wild type Nurr1 protein; and/or a se-  
quence that does not include any Tyr residue in the position corresponding to amino  
15 acid no. 122 of the wild type Nurr1 protein.

A fourth aspect of the invention is a screening method, wherein a protein or a pepti-  
de according to the invention is used as a lead compound to identify substances ca-  
pable of altering, e.g. inhibiting, partially or totally, suppressing, enhancing, resto-  
20 ring etc, the biological effect of said protein or peptide. Accordingly, the substances  
identified by the present method will be useful as medicaments for the treatment of  
psychotic conditions, specifically schizophrenia and/or manic depressive disorder  
and such substances are also encompassed by the present invention. Methods of  
screening, such as high throughput screening etc, are known to the skilled in this fi-  
eld, see e.g. US patent nos 5 763 263 and 5 710 266. In brief, such a method may  
25 e.g. include

- (i) immobilization of the present protein or peptide on a solid support;
- (ii) exposure to a library of candidate substances; and
- (iii) detecting the binding of protein or peptide to candidate.

Further, the present protein or polypeptide may also be used to specifically design a substance binding thereto and capable of inhibition or other alteration of the function thereof.

5 Thus, in a fifth aspect, the invention also relates to methods of treatment as well as to the use of the present proteins or peptides in the manufacture of a medicament. Further, the present proteins or peptides are also useful in diagnosis of schizophrenia and/or manic depressive disease, as the expression thereof in a patient will be an indication of the existence of one of the mutations according to the invention.

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A further aspect of the invention is an antibody which specifically binds to a protein or peptide according to the invention and preferably comprises at least about 10, more preferably at least 20, 40 or 50 or more amino acids. Such an antibody may be used as a medicament in order to inhibit or suppress the biological effect of the product of a mutated gene. Alternatively, the present antibody may be used in the diagnosis of schizophrenia and/or manic depressive disorder. The present antibody may be polyclonal or monoclonal, preferably, it is a monoclonal antibody. It can be humanized or human. The invention also relates to a cell, such as a recombinant cell, e.g. hybridomas or triomas, expressing such an antibody as defined above.

15  
20 Methods of producing polyclonal antibodies are known to those of skill in the art. In brief, an immunogen (antigen), preferably a purified polypeptide, a polypeptide coupled to an appropriate carrier (e.g., GST, keyhole limpet hemocyanin, etc.), or a polypeptide incorporated into an immunization vector such as a recombinant vaccinia virus (*see*, U.S. Patent No. 4,722,848) is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the polypeptide of interest. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the polypeptide is performed where desired (*see*, e.g., Coligan (1991) *Current Protocols in Im-*

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*munology* Wiley/Greene, NY; and Harlow and Lane (1989) *Antibodies: A Laboratory Manual* Cold Spring Harbor Press, NY).

Monoclonal antibodies are prepared from cells secreting the desired antibody. These  
5 antibodies are screened for binding to normal or modified polypeptides, or screened  
for agonistic or antagonistic activity. Specific monoclonal and polyclonal antibodies  
will usually bind with a  $K_D$  of at least about 0.1 mM, more usually at least about 50  
 $\mu$ M, and most preferably at least about 1  $\mu$ M or better. In some instances, it is desir-  
able to prepare monoclonal antibodies from various mammalian hosts, such as mice,  
10 rodents, primates, humans, etc. Description of techniques for preparing such mono-  
clonal antibodies are found in, e.g. Stites *et al.* (eds) *Basic and Clinical Immunology*  
(4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therin;  
Harlow and Lane, *supra*; Goding (1986) *Monoclonal Antibodies: Principles and  
Practice* (2d ed.) Academic Press, New York, NY; and Kohler and Milstein (1975)  
15 *Nature* 256: 495-497.

The pharmaceutical preparations comprising a substance identified by use of the he-  
rein described methods according to the invention, or an antibody as disclosed above,  
according to the invention may be prepared by any standard method according to  
20 standard methods, see e.g. *Remington's Pharmaceutical Sciences*, 16<sup>th</sup> ed. Osol, A  
(ed.) 1980; and Langer, *Science* 249, 1527-1533 (1990).

The peptides, polypeptides and proteins according to the invention may be synthe-  
sized using standard chemical peptide synthesis techniques well known to the skilled  
25 person. For solid phase synthesis, see e.g. Barany and Merrifield, *Solid-Phase Pep-  
tide Synthesis*, in *The Peptides: Analysis, Synthesis, Biology*, vol 2: *Special Methods  
in Peptide Synthesis*.

The present peptides and polypeptides may also be produced using recombinant  
30 DNA technology. Generally, this involves creating a DNA sequence that encodes

the desired peptide, i.e., including one or more of the present mutations, e.g. as a recombinant sequence encoding the present peptide as part of a fusion protein, placing the DNA in an expression cassette under the control of a particular promoter, expressing the peptide in a host and isolating the expressed peptide. In case of a protein, the renaturation thereof may be required. The nucleic acids encoding the peptides or proteins may be expressed in a variety of host cells, including E.coli, other bacterial hosts, yeast, and various higher eucaryotic cells, such as COS, CHO and HeLa cell lines and myeloma cell lines. The gene will be operably linked to appropriate expression control sequences for each host. For E.coli, this includes a promoter such as the T7, trp or lambda promoters, a ribosome binding site and preferably a transcription termination signal. For eucaryotic cells, the control sequences will include a promoter and preferably an enhancer and it may include splice donor and acceptor sequences. The expression systems that may be used for the preparation of the present proteins and peptides include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the present nucleotide sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the present nucleotide sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the present sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the present nucleotide sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

Once expressed, standard procedures may be used for purification, such as ammonium sulphate precipitation, affinity columns, column chromatography, gel elec-

trophoresis and the like. See, generally, R. Scopes, *Protein Purification*, Springer-Verlag, N.Y. (1982), Deutscher, *Methods in Enzymology*, Vol 182: *Guide to protein purification*, Academic Press, Inc., N.Y. (1990).

5 A further aspect of the invention is a cell culture comprised of transgenic cells harbouring one or more of the present mutations for use as a model, e.g. for testing novel pharmaceutically active substances. Such cells may carry one or more of the present mutations on a vector or in the genome. In one embodiment, they are isolated cells that have been mutated spontaneously in a natural environment, while  
10 another embodiment relates to cells produced by recombinant DNA techniques. Such a cell culture will comprise immortalized cells, such as primary nerve cells, COS or HeLa cells etc. Any one of the various methods currently used for the introduction into and amplification of foreign (heterologous) nucleic acid into cultured cells for research purposes as well as for the purpose of overproduction of proteins  
15 of interest for pharmaceutical applications may be used according to the invention. The present cell lines comprise inserted genes or gene fragments as described above. Alternatively, they are derived from a transgenic animal (see e.g. Babinet *et al.*, US patent no. 5 073 490). Transgenic animals are disclosed in further detail below, but a general review of the basic process for the creation of transgenic animals, particularly mice, is found e.g. in Palmiter *et al.*, in "Dramatic Growth of Mice That  
20 Develop From Eggs Microinjected with Metallothionein-Growth Hormone Fusion Genes", *Nature*. Vol. 300, 611-615 (Dec. 1982).

However, mammalian systems are the most preferred. In mammalian host cells, a  
25 number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the nucleotide sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome will result in a recombinant virus that is viable and ca-

pable of expressing the present gene product in infected hosts. (See e.g. Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:3655-3659). Specific initiation signals may also be required for efficient translation of the present inserted nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire Nurr1 gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (See Bittner *et al.*, 1987, *Methods in Enzymol.* 153, 516-544). Further, a purification of the present product is needed. For example, an antibody specific for the expressed peptide or protein may be used. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88, 8972-8976).

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Further, the present invention relates to a transgenic animal, preferably a non-human animal, such as a mammalian, e.g a mouse, comprising a mutated gene or a gene fragment according to the invention, as well as to any further generation of said animal. For a comprehensive instruction on how animal models can be established for human diseases with identified genetic mutations, reference is made to Human Molecular Genetics, BIOS Scientific Publishers Limited, 1996, chapter 19, pp. 507-550, entitled "Studying human gene structure and function and creating animal models of disease", Strachan, T. and Read, A.P. Any technique known in the art may be used to introduce the present gene comprising one or more of the novel mutations into animals to produce the founder lines of transgenic animals. Such techniques

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include, but are not limited to pronuclear microinjection (Hoppe, P.C. and Wagner, T. E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten *et al.*, 1985, *Proc. Natl. Acad. Sci., USA* 82:6148-6152); gene targeting in embryonic stem cells (Thompson *et al.*, 1989, *Cell* 56:313-321); electroporation of embryos (Lo, 1983, *Mol Cell. Biol.* 3:1803-1814); and sperm-mediated gene transfer (Lavitrano *et al.*, 1989, *Cell* 57:717-723); etc. For a review of such techniques, see Gordon, 1989, Transgenic Animals, *Intl. Rev. Cytol.* 115:171-229. Most preferably, the transgenic animal according to the invention is a mouse comprising one or more of the present mutations M1-M3.

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Alternatively, instead of introducing a complete gene or gene fragment into the host, the desired mutation(s) is provoked in a laboratory animal, such as a mouse. This may be accomplished by any mutagenic process, including in vitro-mutagenesis followed by transfer of genomic material into embryonic stem cells or any other mutagenic process, including chemical, biological, radioactive, or ultraviolet treatments.

15

Due to the fact that the domains of the Nurr1 gene surrounding M1, M2 and M3 are highly conserved in mouse and human, see e.g. Ichinose *et al.*, *supra*, the most preferred transgenic animal model according to the invention is a mouse, wherein one or more of the present mutations M1-M3 have been introduced. Thus, for reasons of simplicity in the preparation, the mouse model is an ideal candidate for studies of the present human mutations.

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Methods of transferring genes into the germline, the expression of natural and hybrid genes and phenotypic changes that have occurred in transgenic mice are described by Palmiter and Brinster in *Ann. Rev. Genet.* 20 (1986) 465-499.

30

Accordingly, the present invention also relates to the use of an animal model as defined above for the identification of new drugs. More specifically, such a method of

testing the therapeutic activity of a pharmacological agent on schizophrenia or manic depressive illness comprises administering an effective amount of said pharmacological agent to the mouse model according to the invention and evaluating said agent's effect on said mouse. Any substance identified by use of a model according  
5 to the invention is also within the scope of the invention.

Another aspect of the invention is a method of detecting the presence of a mutation in exon 3 of the Nurr1 gene, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del, which method comprises obtaining a biological sample from e.g. a human subject or an animal model and analyzing said sample for said mutation. More specifically, the biological sample is analyzed by isolating DNA from said sample, amplifying said DNA, hybridising said DNA to a labeled oligonucleotide probe that specifically hybridizes to mutant DNA containing a G as the first base of codon no 97; a G as the second base of codon no  
10 103; or (how do I say this:) altered TACTAC in codon no 121 or 122, or to one or more bases adjacent to said mutation, depending on the method used. Methods of detecting mutations in DNA are e.g. reviewed in U. Landgren, GATA 9, 1992, pp.  
15 3-8.

20 Further, the present mutations in the Nurr1 gene can be detected by either of the following methods (a)-(j), which are described in detail in: (1) *Mutation Detection, A Practical Approach*, R.G.H. Cotton, E. Edkins and S. Forrest (editors), The Practical Approach Series, Oxford University press (1998) and (2) *Finding Mutations, The Basics*, J.R. Hawkins, Oxford University Press (1997).

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(a) Single strand conformation polymorphism analysis (SSCA, also called SSCP), alone or in combination with heteroduplex analysis (HA) is one of the most widely used approaches for mutation detection. (b) Denaturing gradient gel electrophoresis (DGGE) is the name of a whole family of similar methods that are based on the reduction in electrophoretic mobility of a DNA molecule in a dense medium during  
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denaturation. (c) The ribonuclease protection assay or RNase cleavage assay (RPA), the chemical cleavage of mismatch (CMM) assay and mutation detection using T4 endonuclease VII (EMC) are effective methods to detect DNA-DNA or DNA-RNA mismatches caused by mutations. (d) Hybridisation with sequence specific oligonucleotide probes (SSOP) takes advantage of the fact that under stringent conditions, a single basepair mismatch can prevent hybridisation of short complementary oligonucleotide probes. (e) Ligation assays such as the oligonucleotide ligation assay (OLA) comprise further ways of detecting changes in DNA sequences. (f) Direct sequencing, which constitutes one of the most sensitive methods of mutation detection. (g) A rather new method originating from direct sequencing is called minisequencing or solid phase minisequencing. (h) Selective amplification of specific alleles (PASA, also called ASA or ASP) makes it possible to detect mutations already during amplification of the fragment of interest by PCR. (i) With the protein truncation test (PTT), one can identify mutations that induce stop codons in DNA sequence after translation of the sequence into protein. (j) Restriction fragment length polymorphism (RFLP) analysis makes use of endonucleases which recognize and cleave specific nucleotide sequences. Mutations in DNA can either change the sequence so that an endonuclease which cuts the wildtype sequence cannot recognize and cut the mutated sequence, or it can induce a new cleavage site for an endonuclease not cutting the wildtype sequence. In sequences that cannot be distinguished by naturally occurring enzymes, mutations can be analyzed by primer-introduced restriction analysis, a method which alters the sequence surrounding the mutation.

In addition, the invention also relates to a kit for performing any one of the methods described above. Accordingly, essential agents for mutation detection according to the respective method can be combined in single packages as user-friendly kits for easy mutation detection. Such kits may contain reagents and enzymes for amplification of the mutated sites, oligonucleotides hybridizing to DNA sequences around and/or containing the mutations and enzymes for specific cleavage of DNA at mutated sites. Further, the present kits may also include suitable labels. The present in-

vention also relates to the production of any such kit aimed at detecting the mutations M1-M3 identified by the inventors.

Finally, the present invention also relates to methods of gene therapy aimed at correcting one or more of the mutations according to the invention. Techniques for gene therapy are well known to the skilled artisan. For example, WO 93/24640 and WO 95/11984 disclose methods and compositions for *in vivo* gene therapy using nonviral or viral vector technology. In another example, WO 95/06743 discloses a method whereby therapeutic nucleic acid constructs are introduced into a patient's isolated airway epithelial cells via transformation with a viral vector containing a construct. The transformed cells are then administered to the patient. The technology for the various nonviral or viral vectors, that can be applied to cells *in vitro* or injected or applied *in vivo* to patients are likewise well known to the skilled artisan.

15 Detailed description of the drawings

Figure 1 is an overview of the genomic structure of the human Nurr1 gene, distribution of PCR fragments 0-7 and localization of the mutations M1-M3 according to the invention in exon 3. All three mutations are localized within 78 basepairs of PCR fragment 1 comprising the first 495 bases of exon 3. Fragment 0 was only sequenced once in order to design a new primer within the second intron closely adjacent to exon 3. All other fragments (1-7) were used for the sequencing of the coding region in the first 20 Parkinson patients, 20 schizophrenics and 4 controls. After identification of the first mutation, only fragment 1 was sequenced in all further patients.

25 Figure 2 shows autoradiographs of the three heterozygous mutations M1-M3 according to the invention. *a*, M1 (M97V) leads to a double band at the first nucleotide of the codon for amino acid position 97. The arrow points at the wildtype band. *b*, M2 (H103R) exchanges the middle nucleotide in the codon for amino acid 103. The arrow indicates the wildtype. *c*, M3 ( $\Delta Y122$ ) deletes one of the tyrosines at positions 30 121 or 122. It is not possible to tell which three consecutive bands have been dele-

ted. However, the pattern of the 3 basepair shift beginning at the TACTAC sequence must result from a 3 basepair deletion within these six basepairs and the surrounding amino acid sequence is not affected by this shift. Because a reverse primer was used for generating this autoradiograph, the shift occurs in the adjacent sequence 5 above the double tyrosine codons. On the right, the wildtype (wt) sequence is shown for comparison.

Figure 3 shows in table 1 the primers used to amplify the PCR fragments 0 to 7. Because no intronic sequences were known when the present study was initiated, primers are located in exons and almost all fragments contain intron sequence. More 10 specifically, fragment no. 0 comprises a part of the second (non-coding) exon, intron 1 (complete) and 2 bases of exon 3; in fragment no. 1, the forward primer is located in the second intron, immediately adjacent to the border to the third exon, and this fragment contains the first 513 bases of exon 3; fragment no. 2 is the second half of exon 3, both primers being located within coding sequence; fragment no. 3 is 15 the rest of exon 3, complete intron 3 and major part of exon 4; fragment no.4 comprises exon 4, intron 4 and exon 5; fragment no.5 comprises exon 5, intron 5 and exon 6; fragment no.6 comprises exon 6, intron 6 and exon 7; and fragment no.7 comprises exon 7, intron 7 and exon 8 including 34 bases of 3' untranslated region.

Figure 4 provides by table 2 clinical descriptions of the three heterozygous carriers 20 of mutations M1 to M3 according to the invention. In table 2, the following abbreviations are used: MD: Major depression; SAP: Separation Anxiety Disorder; ADHD: Attention Deficit/Hyperactivity Disorder. More specifically, the history of the female heterozygous carrier of M1 is an onset with auditory hallucinations, later displayed delusions of reference, paranoid delusions, verbal auditory hallucinosis, 25 visual and tactile hallucinosis, thought insertion and thought "broadcast", as well as flat and sometimes inappropriate affects. She responded well to antipsychotic treatment but experienced relapses after discontinuation of the antipsychotic medication. She is currently on continuous antipsychotic medication and has been free from psychotic episodes for several years. Her family history was as follows: The paternal grandmother's sister had been treated at a mental hospital, the cause of illness 30

unknow. The history of the M2 carrier includes a first hospital admission after 5 months of expansive and irritable mood, decreased need for sleep, distractability, and excessive involvement in pleasurable activities. The end of this period also included psychotic symptoms: verbal mood-congruent auditory hallucinations, delusions of reference, grandiose delusions, incoherence, and disorganized behaviour.

5 Treatment was performed with antipsychotic medication and discharge to an outpatient department. Rehospitalization took place a month later due to depressed mood, weight gain, loss of energy, feeling of worthlessness, difficulties to concentrate, and recurrent thought of death. No psychotic symptoms occurred during later manic and

10 depressive episodes. She has currently been treated with lithium for more than four years without relapses. The family history of the M2 carrying patient was as follows: The patient reported no major psychiatric disturbances in the family history. However, the paternal grandfather was said to be a confidence trickster. The history of the M3 carrier includes two episodes of extended depressed mood and anhedonia

15 (ages 6-7 and 10-11). Auditory hallucinations were experienced since the second episode. Hallucinations and delusions occur regularly and are independent of mood state. Intermittent history of obsessions and compulsions since age of 6. Frequent episodes of illogical thinking and neologisms and short periods of incoherent speech. The family history of the M3 carrying patient was as follows: The mother re-

20 ported a few occasional (olfactory, visual) hallucinatory episodes but no further symptoms.

### Experimental

25 The examples below are given merely to illustrate the present invention and are not intended to limit the scope of the invention as defined by the appended claims. All references given below or elsewhere in the present specification are included herein by reference.

Example 1: Identification of mutationsMethodsGeneral

By direct sequencing of genomic DNA, one missense mutation and one deletion of three basepairs in the third exon of Nurr1 were found in two schizophrenic patients. One missense mutation was found in the same exon in an individual with manic depressive disorder. None of these mutations were present in patients with Parkinson's disease or control DNA material of normal populations.

- 10 The entire coding region of the Nurr1 gene was first sequenced in 20 patients with Parkinson's disease, 20 patients with schizophrenia and 4 healthy control individuals. The primers used for PCR amplification of the DNA fragments shown in figure 1 are summarized in table 1. One deletion of three basepairs ( $\Delta Y122$ , fig 1 and 2) was identified in one childhood-onset schizophrenic individual originating from the USA. This mutation (M3) was located in fragment 1 which covers about the first half of the N-terminal domain. This mutation was not found when fragment 1 was sequenced in 100 healthy individuals from the USA, nor were any other mutations found in the same fragment in this material. As mutations are frequently clustered in coding regions of disease-causing genes, the present sequencing efforts were continued by focusing on fragment 1. This fragment was sequenced in 60 Swedish controls, 50 additional Swedish Parkinson patients, 17 additional American schizophrenics, 135 additional Swedish schizophrenics and 29 manic depressed patients from the USA and Sweden. One missense mutation (H103R; M2 in the figures) was identified in a Swedish schizophrenic patient and another missense mutation (M97V; M1 in the figures) was identified in a Swedish patient with manic-depressive illness with psychotic symptoms. All three mutations were absent from all other DNA samples and are therefore unlikely to be polymorphic variants of the human Nurr1 gene. Notably, no mutations were found in the Parkinson DNA samples. Attempts to obtain DNA samples from relatives of the three mutation carriers were only moderately successful. Only in the childhood onset case (M3) was it

possible to obtain parental DNA. The mother was a healthy carrier of the mutation, but no further family history could be obtained in this kindred. The medical and family histories of all three patients are summarized in relation to table 2, figure 4. Interestingly, all three mutations cluster in evolutionary conserved amino acids in close vicinity to each other in the N-terminal region of Nurr1, which might indicate a common effect on Nurr1 function. Preliminary results from *in vitro* mutagenesis experiments indicate that transcriptional activity mediated by Nurr1 monomer binding to the Nurr1 binding response element is not severely affected by any of the three mutations (data not shown). More complex assays measuring Nurr1 heterodimerization with RXR, Nurr1 homodimerization and interaction of Nurr1 with other activating or silencing factors are needed to elucidate possible functional consequences of the mutations. Because the Nurr1 gene is evolutionary highly conserved and all amino acids affected by the mutations are identical in mice, generation of transgenic mice carrying the novel mutations according to the invention provides additional opportunities to understand how Nurr1 function is affected. The generation of such mice will help elucidating one pathway for the development of schizophrenia and/or bipolar affective disorder and thus will also help to find further genetic and environmental etiological factors for these diseases.

## 20 Patients

All schizophrenic and bipolar affective disorder patients fulfilled the DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, Third Revised Edition. Copyright 1987 American Psychiatric Association) or DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Copyright 1994 American Psychiatric Association) diagnostic criteria. Parkinson patients were diagnosed according to the "Brain bank clinical criteria" for idiopathic Parkinson's disease (Daniel, S.E. & Lees, A.J. Parkinson's Disease Society Brain Bank, London: overview and research. *J. Neural Transm. Suppl.* 39, 165-172 (1993)) except that three cases had more than one affected relative but were still included. DNA samples from bipolar affective disorder patients were purchased from the Coriell Cell Repository (Cam-

den, USA). While all schizophrenic and Parkinson patients involved in this study were unrelated individuals, patients with bipolar affective disorder belonged to the Coriell families number 811 (n=3), 812 (n=2), 823 (n=5), 830 (n=4), 834 (n=1) 835 (n=1), 884 (n=5), 888 (n=1), 893 (n=2) and 1075 (n=2). Three manic depressed patients from Sweden were also included in the material as presumed controls or schizophrenic patients. At the present study they turned out to fulfill the DSM-III-R criteria for bipolar affective disorder.

#### DNA sequencing

10 DNA was extracted from whole blood according to standard protocols. The genomic structure of the human Nurr1 gene was deduced from the homologous mouse Nurr1 gene, and primers covering the second exon and the entire coding region were designed (table 1, fig 3). The numbering of the nucleotides and amino acids employed herein follows the initial publication of the human Nurr1 (*NOT*) mRNA sequence (Mages *et al.*, *supra*). The sequence of the second intron was determined by sequencing of fragment 0, and one additional forward primer was designed located in the second intron (see table 1, figure 3, and figure 1). Polymerase chain reaction (PCR) was carried out using Taq DNA polymerase (SIGMA). 35 cycles were run at 94°C for 40 seconds, 56°C for 45 seconds and 72°C for one minute. After PCR, the 15 samples underwent electrophoresis on 1% low-melting agarose gels and were visualized using UV-transillumination after ethidiumbromide staining. DNA was extracted from gel slices (PCR preps DNA purification kit, SDS) and DNA fragments were sequenced (Thermo Sequenase radiolabeled terminator cycle sequencing kit, Amersham). The reaction products were run on 6% acrylamid sequencing gels (National Diagnostics) and visualized by autoradiography.

#### Results

30 The present invention shows that two out of 187 patients with schizophrenia and one out of 29 patients with bipolar affective disorder carried unique mutations clustered in the area of the N-terminal domain of the Nurr1 gene. Even though the fre-

quency of mutated Nurr1 determined according to the invention may appear low (about one percent for schizophrenia and three percent for bipolar disorder), it is perfectly in line with the multifactorial origin of these diseases, with a number of different gene defects remaining to be identified explaining a fraction of the total  
5 number of patients each.

Example 2: Function of identified mutations

10 Because the rarity of molecular variants of *NURR1* does not exclude their role in the pathogenesis of psychiatric disease in those individuals where they were identified, functional assays were set up in order to elucidate if any of the three clustered mutations might affect *NURR1* function. The following description of results from such functional studies is adapted from the manuscript entitled "NURR1 Mutations  
15 in Cases of Schizophrenia and Manic Depressive Disorder" by authors Silvia Buer-  
venich, Andrea Carmine, Mariette Arvidsson, Fengqing Xiang, Zhiping Zhang, Olof  
Sydow, Erik G. Jönsson, Göran C. Sedvall, Sherry Leonard, Randal G. Ross, Robert  
Freedman, Kodavali V. Chowdari, Vishwajit L. Nimgaonkar, Thomas Perlmann,  
Maria Anvret, and Lars Olson. The cited work is currently in press in the American  
20 Journal of Medical Genetics, Neuropsychiatry Section.

In-vitro expression assay

Human *NURR1* cDNA sequence was cloned into the expression vector pCMX [Umesono et al., 1991], and expression vectors for the mutants were generated by site-directed mutagenesis (GeneEditor In Vitro Site-Directed Mutagenesis System, Promega). A double stranded NurRE [Philips et al., 1997] DNA fragment was generated by annealing the primers 5'-AGC TTG TGA TAT TTA CCT CCA AAT GCC AG-3' and 5'-AGC TCT GGC ATT TGG AGG TAA ATA TCA CA-3'. A luciferase reporter plasmid containing three tandem NurRE sites was generated by ligating  
25 the annealed fragments upstream of the herpes simplex thymidine kinase promoter  
30

fused to the luciferase gene. Human embryonic kidney (HEK)-293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS). Transfections were performed in 24-well plates by the calcium phosphate method. Briefly, cells were seeded one day prior to transfection. Cells in each well were treated with 100ng of the indicated expression vectors, 100ng of reporter plasmid and 200ng of reference CMX- $\beta$ gal plasmid containing the  $\beta$ -galactosidase gene and CMX-PL1 as carrier DNA up to 500ng of total DNA. Cells were exposed to calcium phosphate precipitate for 12-16h and washed with PBS and then fresh medium was added. The cells were harvested and lysed after 36h incubation. Extracts were assayed for luciferase and  $\beta$ -galactosidase activity in a microplate luminometer/photometer reader (Lucy-1, Anthos). All luciferase activities were normalized to  $\beta$ -galactosidase activity.

NURR1 transcriptional activity was measured using human embryonic kidney (HEK)-293 cells monitoring NURR1 homodimer binding to NurRE [Philips et al., 1997]. Each experiment was carried out using two independent clones for the wild-type and mutated vectors, respectively, and activity was measured in at least four separate wells for each clone. A significant reduction (30-40%) of transcriptional activity of mutated NURR1 homodimers was found. This reduction was strikingly similar in all three mutated clones and is consistent with the present finding that the three mutations are clustered in a region of NURR1 which is critically important for transcriptional activation. The average activity and s.e.m. of mutated NURR1 in percent of wildtype activity in all experiments carried out were  $\Delta Y122$ :  $0.645 \pm 0.024$ , n=87; H103R:  $0.608 \pm 0.036$ , n=87; and M97V:  $0.661 \pm 0.046$ , n=54.

25

The facts that (1) the three mutations were clustered within 78 basepairs of the N-terminal domain, (2) they all lead to impaired NURR1 transcriptional activity in an *in-vitro* bioassay, and (3) except for the three mutations this region of the *NURR1* gene was totally conserved at the DNA level in our entire material of 620 individu-

30

als (1240 alleles) suggest that the *NURR1* gene does constitute an interesting gene for mutation screening in diseases with an involvement of the dopamine system.

Figure 3

Table 1

Fragment number	Description	Primer sequences
0	Part of the second (noncoding) exon, intron 1 (complete) and 2 bases of exon 3	F - GGAGATTGGACAGGCTGGAC R - TGCGCCTGAACACAAGGCAT
1	The forward primer is located in the second intron, immediately adjacent to the border to the third exon. This fragment contains the first 513 bases of exon 3	F - TTATCACCCCTGTTCAATTCC R - GAGACTGGCGTTTCCTCT
2	Second half of exon 3. Both primers are located within coding sequence	F - TGCCGCACTCCGGTGGTTACTACA R - GCCCTCACAGGTGCGCACGCCGTA
3	Rest of exon 3, complete intron 3 and major part of exon 4	F - CACGCGTCTCAGCTGCTCGACAC R - CTTCTTGACCATCCAACAGCCA
4	Exon 4, intron 4 and exon 5	F - CGCACAGTGCAAAAAATGCAA R - CCTGGAATAGTCCAGGCTGG
5	Exon 5, intron 5 and exon 6	F - TGGTCGCACAGACAGTTA R - GCTAATCGAAGGACAAACAG
6	Exon 6, intron 6 and exon 7	F - TTCCAGGCGAACCCCTGACTA R - ACCATAGCCAGGGCAGCAAT
7	Exon 7, intron 7 and exon 8 including 34 bases of 3' untranslated region	F - TCCAACCCAGTGGAGGGTAA R - ATTCCAGTTCCCTTGAAGTGC

Figure 4

Table 2

Mutation number	Diagnosis	Country of origin	Age of onset	Prominent symptoms	Affected family members
M1	Schizophrenia	Sweden	26	Auditory hallucinations, delusions of reference, and paranoid delusions.	Paternal grandmother's sister
M2	Bipolar disorder with psychotic features	Sweden	21	Auditory hallucinations, delusions of reference, incoherence and depressed mood.	Paternal grandfather
M3	MD (past); Childhood-onset schizophrenia, SAP, ADHD (current)	USA	6	Extended depressed mood, anhedonia, auditory hallucinations and delusions.	Not known

CLAIMS

1. An isolated Nurr1 gene including one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del), or a functional fragment or variant thereof.
- 5 2. A fragment according to claim 1, which comprises the exons of the Nurr1 gene.
3. A fragment according to claim 1 or 2, which comprises exon 3 of the Nurr1 gene.
4. A fragment according to any one of claims 1-3, which comprises the mutation Met97Val.
- 10 5. A fragment according to any one of claims 1-3, which comprises the mutation His103Arg.
6. A fragment according to any one of claims 1-3, which comprises the mutation Tyr121del or Tyr122del.
7. A nucleic acid capable of specifically hybridising to a gene or a fragment according to any one of claims 1-6.
- 15 8. A vector comprising a nucleic acid according to any one of claim 1-7.
9. A recombinant cell comprising a vector according to claim 8.
10. An isolated cell carrying one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del) in its genome.
- 20 11. A cell culture comprising cells according to claim 9 or 10, which cells are immortalized cells, such as mammalian cells.
12. A protein or a peptide encoded by a gene or a gene fragment according to any one of claims 1-7.
13. A protein or peptide according to claim 12, which includes a Val residue in the 25 position corresponding to amino acid no. 97 of the wild type Nurr1 protein.
14. A protein or peptide according to claim 12, which includes an Arg residue in the position corresponding to amino acid no. 103 of the wild type Nurr1 protein.
15. A protein or peptide according to claim 12, which does not include any Tyr residue in the position corresponding to amino acid no. 121 or 122 of the wild type 30 Nurr1 protein.

16. A method of screening for pharmaceutically active substances, wherein a nucleotide according to any one of claims 1-7 or a protein or peptide according to any one of claims 12-15 is used as a lead compound to identify substances capable of altering the biological effect of said nucleotide, or protein or peptide.
- 5      17. A substance identified by use of a screening method according to claim 16.
18. An antibody raised against a protein or peptide according to any one of claims 12-15.
19. A transgenic, non-human animal, such as a mouse or a rat, comprising a gene or a gene fragment according to any one of claims 1-7.
- 10     20. A transgenic mouse comprising a mutation in the chromosome corresponding to the human chromosome 2q22-23 of said mouse, or an ancestor thereof, introduced at an embryonic stage such that said transgene replaces an endogenous allele resulting in said mutation, which transgenic mouse comprises one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del).
- 15     21. A method of screening for pharmaceutically active substances, wherein an animal according to claim 19 or 20 is used as a lead to identify substances capable of altering the biological effect of a nucleotide according to any one of claims 1-7 and/or a protein or peptide according to any one of claims 12-15.
- 20     22. A substance identified by use of a screening method according to claim 21.
23. An antibody according to claim 18 or a substance according to claim 17 or 22 for use as a medicament.
24. Use of an antibody according to claim 18 or a substance according to claim 17 or 22 in the manufacture of a medicament for the treatment and/or prevention of psychotic conditions, such as schizophrenia and/or manic depressive disorder.
- 25     25. A pharmaceutical preparation comprising an antibody according to claim 18 or a substance according to claim 17 or 22 together with a pharmaceutically acceptable carrier.
26. A method of detecting the presence of a mutation in exon 3 of the Nurr1 gene, which mutation is selected from the group consisting of Met97Val, His103Arg,

Tyr121del and Tyr122del, said method comprising obtaining a biological sample from a mammalian, such as a human, subject and analyzing said sample for said mutation.

27. A method according to claim 26, wherein the biological sample is analyzed by isolating DNA from said sample, amplifying said DNA, hybridising said DNA to a labeled oligonucleotide probe that specifically hybridizes to mutant DNA containing a G as the first base of codon no 97; a G as the second base of codon no 103; or a deleted TAC in codon no 121 or 122, or to the close vicinity of said DNA.

10 28. A kit for performing the method according to claim 26 or 27, which kit comprises:

- (a) reagents for amplification of one or more of the mutated sites; and/or
- (b) enzymes for specific cleavage of DNA; and
- (c) optionally suitable labels.

15 29. A method of treating and/or preventing a condition associated with schizophrenia and/or manic depression in a patient in need of therapy, wherein a mutation in exon 3 of the Nurr1 gene is corrected, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del.

30. A method according to claim 29, wherein the DNA of one or more of said mutations is replaced by DNA having the native, non-mutated base sequence using a vector suitable for transfecting the patient.

20 31. A method according to claim 29, wherein cells comprising the native, non-mutated base sequence in the positions corresponding to one or more of said mutations are introduced in said patient.

**AMENDED CLAIMS**

[received by the International Bureau on 24 November 2000 (24.11.00);  
original claims 17 and 22 amended;  
remaining claims unchanged (3 pages)]

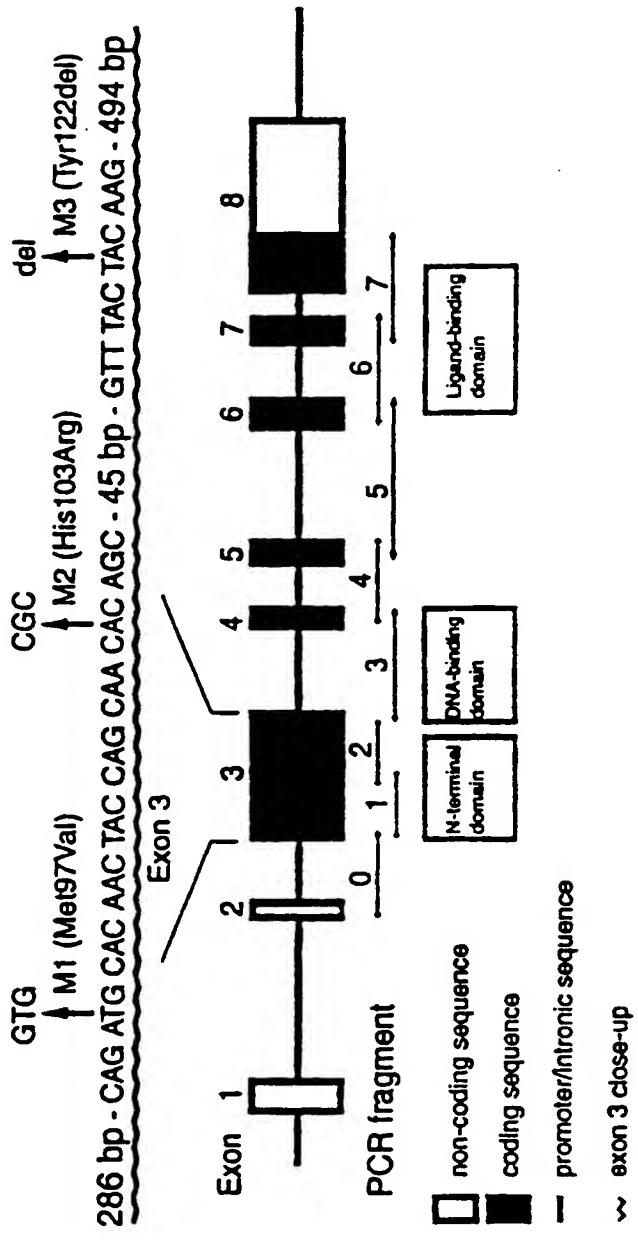
1. An isolated Nurr1 gene including one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del), or a functional fragment or variant thereof.
2. A fragment according to claim 1, which comprises the exons of the Nurr1 gene.
3. A fragment according to claim 1 or 2, which comprises exon 3 of the Nurr1 gene.
4. A fragment according to any one of claims 1-3, which comprises the mutation Met97Val.
5. A fragment according to any one of claims 1-3, which comprises the mutation His103Arg.
6. A fragment according to any one of claims 1-3, which comprises the mutation Tyr121del or Tyr122del.
7. A nucleic acid capable of specifically hybridising to a gene or a fragment according to any one of claims 1-6.
8. A vector comprising a nucleic acid according to any one of claim 1-7.
9. A recombinant cell comprising a vector according to claim 8.
10. An isolated cell carrying one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del) in its genome.
11. A cell culture comprising cells according to claim 9 or 10, which cells are immortalized cells, such as mammalian cells.
12. A protein or a peptide encoded by a gene or a gene fragment according to any one of claims 1-7.
13. A protein or peptide according to claim 12, which includes a Val residue in the position corresponding to amino acid no. 97 of the wild type Nurr1 protein.
14. A protein or peptide according to claim 12, which includes an Arg residue in the position corresponding to amino acid no. 103 of the wild type Nurr1 protein.
15. A protein or peptide according to claim 12, which does not include any Tyr residue in the position corresponding to amino acid no. 121 or 122 of the wild type Nurr1 protein.

16. A method of screening for pharmaceutically active substances, wherein a nucleotide according to any one of claims 1-7 or a protein or peptide according to any one of claims 12-15 is used as a lead compound to identify substances capable of altering the biological effect of said nucleotide, or protein or peptide.
17. A method for the production of a pharmaceutical composition comprising the method according to claim 16 and furthermore mixing the substance identified with a pharmaceutically acceptable carrier.
18. An antibody raised against a protein or peptide according to any one of claims 12-15.
19. A transgenic, non-human animal, such as a mouse or a rat, comprising a gene or a gene fragment according to any one of claims 1-7.
20. A transgenic mouse comprising a mutation in the chromosome corresponding to the human chromosome 2q22-23 of said mouse, or an ancestor thereof, introduced at an embryonic stage such that said transgene replaces an endogenous allele resulting in said mutation, which transgenic mouse comprises one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del).
21. A method of screening for pharmaceutically active substances, wherein an animal according to claim 19 or 20 is used as a lead to identify substances capable of altering the biological effect of a nucleotide according to any one of claims 1-7 and/or a protein or peptide according to any one of claims 12-15.
22. A method for the production of a pharmaceutical composition comprising the method according to claim 21 and furthermore mixing the substance identified with a pharmaceutically acceptable carrier.
23. An antibody according to claim 18 or a substance according to claim 17 or 22 for use as a medicament.
24. Use of an antibody according to claim 18 or a substance according to claim 17 or 22 in the manufacture of a medicament for the treatment and/or prevention of psychotic conditions, such as schizophrenia and/or manic depressive disorder.

25. A pharmaceutical preparation comprising an antibody according to claim 18 or a substance according to claim 17 or 22 together with a pharmaceutically acceptable carrier.
26. A method of detecting the presence of a mutation in exon 3 of the *Nurr1* gene, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del, said method comprising obtaining a biological sample from a mammalian, such as a human, subject and analyzing said sample for said mutation.
27. A method according to claim 26, wherein the biological sample is analyzed by isolating DNA from said sample, amplifying said DNA, hybridising said DNA to a labeled oligonucleotide probe that specifically hybridizes to mutant DNA containing a G as the first base of codon no 97; a G as the second base of codon no 103; or a deleted TAC in codon no 121 or 122, or to the close vicinity of said DNA.
28. A kit for performing the method according to claim 26 or 27, which kit comprises:
  - (a) reagents for amplification of one or more of the mutated sites; and/or
  - (b) enzymes for specific cleavage of DNA; and
  - (c) optionally suitable labels.
29. A method of treating and/or preventing a condition associated with schizophrenia and/or manic depression in a patient in need of therapy, wherein a mutation in exon 3 of the *Nurr1* gene is corrected, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del.
30. A method according to claim 29, wherein the DNA of one or more of said mutations is replaced by DNA having the native, non-mutated base sequence using a vector suitable for transfecting the patient.
31. A method according to claim 29, wherein cells comprising the native, non-mutated base sequence in the positions corresponding to one or more of said mutations are introduced in said patient.

1/2

Figure 1



2/2

Figure 1

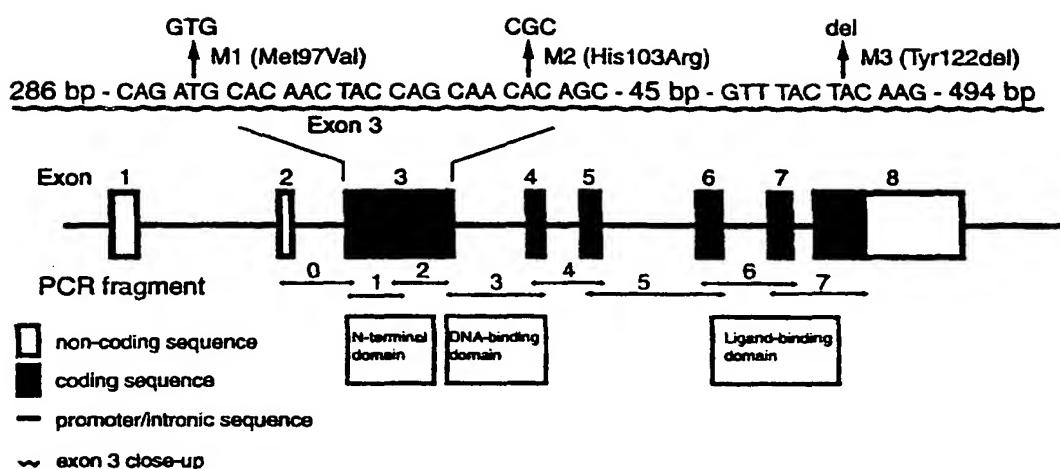
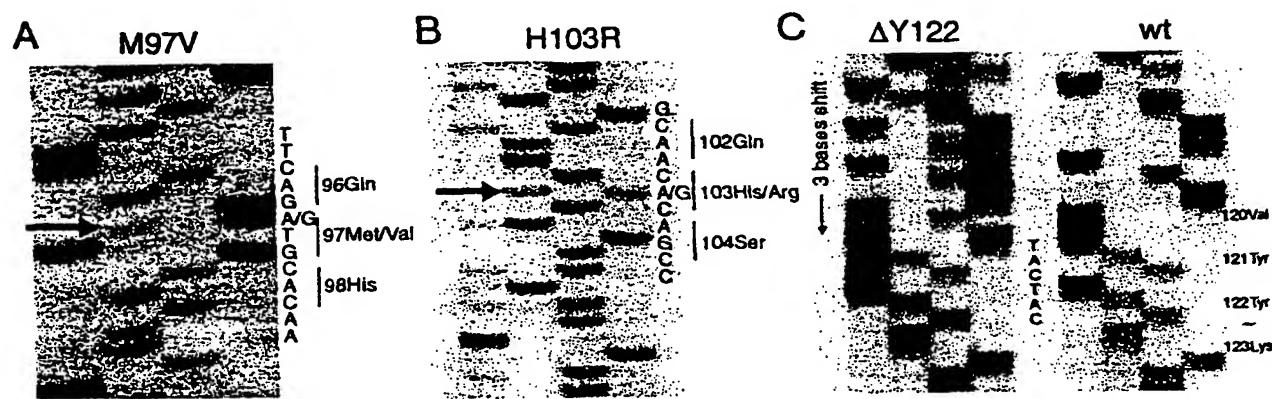


Figure 2



## PENT COOPERATION TREATY

PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

REC'D 17 OCT 2001  
WIPO PCT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference <b>55827-60444</b>	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. <b>PCT/SE00/01380</b>	International filing date (day/month/year) <b>29.06.2000</b>	Priority date (day/month/year) <b>30.06.1999</b>
International Patent Classification (IPC) or national classification and IPC7 <b>C12N 15/00, C01K 14/705, A61K 39/395, A61K 67/027</b>		
Applicant <b>Karolinska Innovations AB et al</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

- I  Basis of the report
- II  Priority
- III  Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV  Lack of unity of invention
- V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI  Certain documents cited
- VII  Certain defects in the international application
- VIII  Certain observations on the international application

Date of submission of the demand <b>29.01.2001</b>	Date of completion of this report <b>08.10.2001</b>
Name and mailing address of the IPEA/SE  Patent- och registreringsverket Box: 5055 S-192 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer  <b>Fernando Farieta/BS</b> Telephone No. 08-782 25 00

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE00/01380

## I. Basis of the report

## 1. With regard to the elements of the international application:\*

 the international application as originally filed the description:

pages 1-26 , as originally filed

pages \_\_\_\_\_, filed with the demand

pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_

 the claims:

pages \_\_\_\_\_, as originally filed

pages \_\_\_\_\_, as amended (together with any statement) under article 19

pages \_\_\_\_\_, filed with the demand

pages 1-4 , filed with the letter of 2001-09-04

 the drawings:

pages 1-3 , as originally filed

pages \_\_\_\_\_, filed with the demand

pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_

 the sequence listing part of the description:

pages \_\_\_\_\_, as originally filed

pages \_\_\_\_\_, filed with the demand

pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_

## 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

 the language of a translation furnished for the purposes of international search (under Rule 23.1(b)). the language of publication of the international application (under Rule 48.3(b)). the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

 contained in the international application in written form. filed together with the international application in computer readable form. furnished subsequently to this Authority in written form. furnished subsequently to this Authority in computer readable form. The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished. The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.4.  The amendments have resulted in the cancellation of: the description, pages \_\_\_\_\_ the claims, Nos. \_\_\_\_\_ the drawings, sheet/fig \_\_\_\_\_5.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**

International application No.

PCT/SE00/01380

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

 the entire international application, claims Nos. 29-31

because:

 the said international application, or the said claims Nos. 29-31relate to the following subject matter which does not require an international preliminary examination (*specify*):

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

the description, claims or drawings (*indicate particular elements below*) or said claims Nos. \_\_\_\_\_  
are so unclear that no meaningful opinion could be formed (*specify*):

the claims, or said claims Nos. \_\_\_\_\_ are so inadequately supported  
by the description that no meaningful opinion could be formed.

no international search report has been established for said claims Nos. \_\_\_\_\_

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

the written form has not been furnished or does not comply with the standard.

the computer readable form has not been furnished or does not comply with the standard.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE00/01380

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

## 1. Statement

Novelty (N)	Claims Claims	<u>1-16, 18-21, 26-28 and part. 23-25</u>	YES NO
Inventive step (IS)	Claims Claims	<u>1-16, 18-21, 26-28 and part. 23-25</u>	YES NO
Industrial applicability (IA)	Claims Claims	<u>1-31</u>	YES NO

## 2. Citations and explanations (Rule 70.7)

During the search, the following documents have been found:

D1: Proc. Natl. Acad. Sci. USA, Volume 95; March 1998,  
 Odila Saucedo-Cardenas et al; " Nurrl essential for the  
 induction of the Dopaminergic phenotype and the survival  
 of ventral mesenphalic late dopaminergic  
 Precursor neurons", Page 4013-4018.

D2: Science, Volume 276, April 1997,  
 Rolf Zetterström et al, "Dopamine neuron Agenesis in  
 Nurrl-Deficient mice", Page 248-580.

D3: US 5783680 A.

Document D1, D2 and D3 are considered to constitute the state of the art. Nurrl is a member of the nuclear receptor superfamily of transcription factors that is expressed predominantly in the central nervous system including developing and mature dopaminergic neurons.

The amended claims filed with letter of 2001-09-04 differ from the claims as originally filed in the removal of the terms "functional fragment" or "variant thereof", in claim 1.

The invention according to amended claims 1-16, 18-21, 26-28 and partially 23-25, is thus novel and is considered to involve an inventive step.

04-09-2001

CLAIMS

1. An isolated Nurrl gene including one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del).
2. A fragment according to claim 1, which comprises the exons of the Nurrl gene.
3. A fragment according to claim 1 or 2, which comprises exon 3 of the Nurrl gene.
4. A fragment according to any one of claims 1-3, which comprises the mutation Met97Val.
5. A fragment according to any one of claims 1-3, which comprises the mutation His103Arg.
6. A fragment according to any one of claims 1-3, which comprises the mutation Tyr121del or Tyr122del.
7. A nucleic acid capable of specifically hybridising to a gene or a fragment according to any one of claims 1-6.
8. A vector comprising a nucleic acid according to any one of claim 1-7.
9. A recombinant cell comprising a vector according to claim 8.
10. An isolated cell carrying one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del) in its genome.
11. A cell culture comprising cells according to claim 9 or 10, which cells are immortalised cells, such as mammalian cells.
12. A protein or a peptide encoded by a gene or a gene fragment according to any one of claims 1-7.
13. A protein or peptide according to claim 12, which includes a Val residue in the position corresponding to amino acid no. 97 of the wild type Nurrl protein.
14. A protein or peptide according to claim 12, which includes an Arg residue in the position corresponding to amino acid no. 103 of the wild type Nurrl protein.
15. A protein or peptide according to claim 12, which does not include any Tyr residue in the position corresponding to amino acid no. 121 or 122 of the wild type Nurrl protein.

04-09-2001

16. A method of screening for pharmaceutically active substances, wherein a nucleotide according to any one of claims 1-7 or a protein or peptide according to any one of claims 12-15 is used as a lead compound to identify substances capable of altering the biological effect of said nucleotide, or protein or peptide.

17. A method for the production of a pharmaceutical composition comprising the method according to claim 16 and furthermore mixing the substance identified with a pharmaceutically acceptable carrier.

18. An antibody raised against a protein or peptide according to any one of claims 12-15.

19. A transgenic, non-human animal, such as a mouse or a rat, comprising a gene or a gene fragment according to any one of claims 1-7.

20. A transgenic mouse comprising a mutation in the chromosome corresponding to the human chromosome 2q22-23 of said mouse, or an ancestor thereof, introduced at an embryonic stage such that said transgene replaces an endogenous allele resulting in said mutation, which transgenic mouse comprises one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del).

21. A method of screening for pharmaceutically active substances, wherein an animal according to claim 19 or 20 is used as a lead to identify substances capable of altering the biological effect of a nucleotide according to any one of claims 1-7 and/or a protein or peptide according to any one of claims 12-15.

22. A method for the production of a pharmaceutical composition comprising the method according to claim 21 and furthermore mixing the substance identified with a pharmaceutically acceptable carrier.

23. An antibody according to claim 18 or a substance according to claim 17 or 22 for use as a medicament.

24. Use of an antibody according to claim 18 or a substance according to claim 17 or 22 in the manufacture of a medicament

04-09-2001

for the treatment and/or prevention of psychotic conditions, such as schizophrenia and/or manic depressive disorder.

25. A pharmaceutical preparation comprising an antibody according to claim 18 or a substance according to claim 17 or 22 together with a pharmaceutically acceptable carrier.

26. A method of detecting the presence of a mutation in exon 3 of the Nurrl gene, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del, said method comprising obtaining a biological sample from a mammalian, such as a human, subject and analysing said sample for said mutation.

27. A method according to claim 26, wherein the biological sample is analysed by isolating DNA from said sample, amplifying said DNA, hybridising said DNA to a labeled oligonucleotide probe that specifically hybridises to mutant DNA containing a G as the first base of codon no 97; a G as the second base of codon no 103; or a deleted TAC in codon no 121 or 122, or to the close vicinity of said DNA.

28. A kit for performing the method according to claim 26 or 27, which kit comprises:

- (a) reagents for amplification of one or more of the mutated sites; and/or
- (b) enzymes for specific cleavage of DNA; and
- (c) optionally suitable labels.

29. A method of treating and/or preventing a condition associated with schizophrenia and/or manic depression in a patient in need of therapy, wherein a mutation in exon 3 of the Nurrl gene is corrected, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del.

30. A method according to claim 29, wherein the DNA of one or more of said mutations is replaced by DNA having the native, non-mutated base sequence using a vector suitable for transfecting the patient.

04-09-2001

31. A method according to claim 29, wherein cells comprising the native, non-mutated base sequence in the positions corresponding to one or more of said mutations are introduced in said patient.

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 55827-60444	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE00/01380	International filing date (day/month/year) 29.06.2000	Priority date (day/month/year) 30.06.1999
International Patent Classification (IPC) or national classification and IPC7 C12N 15/00, C01K 14/705, A61K 39/395, A61K 67/027		
Applicant Karolinska Innovations AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

- I  Basis of the report
- II  Priority
- III  Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV  Lack of unity of invention
- V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI  Certain documents cited
- VII  Certain defects in the international application
- VIII  Certain observations on the international application

Date of submission of the demand 29.01.2001	Date of completion of this report 08.10.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box: 5055 S-101 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer Fernando Farieta/BS Telephone No. 08-782 25 00

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE00/01380

## I Basis of the report

1. With regard to the elements of the international application:<sup>\*</sup> the international application as originally filed the description:

pages 1-26 , as originally filed

pages , filed with the demand

pages , filed with the letter of \_\_\_\_\_

 the claims:

pages , as originally filed

pages , as amended (together with any statement) under article 19

pages , filed with the demand

pages 1-4 , filed with the letter of 2001-09-04

 the drawings:

pages 1-3 , as originally filed

pages , filed with the demand

pages , filed with the letter of \_\_\_\_\_

 the sequence listing part of the description:

pages , as originally filed

pages , filed with the demand

pages , filed with the letter of \_\_\_\_\_

## 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_

which is:

 the language of a translation furnished for the purposes of international search (under Rule 23.1(b)). the language of publication of the international application (under Rule 48.3(b)). the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

 contained in the international application in written form. filed together with the international application in computer readable form. furnished subsequently to this Authority in written form. furnished subsequently to this Authority in computer readable form. The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished. The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.4.  The amendments have resulted in the cancellation of: the description, pages \_\_\_\_\_ the claims, Nos. \_\_\_\_\_ the drawings, sheet/fig \_\_\_\_\_5.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under ~~item 4~~ and annexed to this report.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE00/01380

## III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

 the entire international application, claims Nos. 29-31

because:

 the said international application, or the said claims Nos. 29-31relate to the following subject matter which does not require an international preliminary examination (*specify*):

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

the description, claims or drawings (*indicate particular elements below*) or said claims Nos. \_\_\_\_\_  
are so unclear that no meaningful opinion could be formed (*specify*):

the claims, or said claims Nos. \_\_\_\_\_  
are so inadequately supported  
by the description that no meaningful opinion could be formed.

no international search report has been established for said claims Nos. \_\_\_\_\_

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

the written form has not been furnished or does not comply with the standard.

the computer readable form has not been furnished or does not comply with the standard.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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International application No.

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## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

## 1. Statement

Novelty (N)	Claims Claims	<u>1-16, 18-21, 26-28 and part. 23-25</u>	YES NO
Inventive step (IS)	Claims Claims	<u>1-16, 18-21, 26-28 and part. 23-25</u>	YES NO
Industrial applicability (IA)	Claims Claims	<u>1-31</u>	YES NO

## 2. Citations and explanations (Rule 70.7)

During the search, the following documents have been found:

D1: Proc. Natl. Acad. Sci. USA, Volume 95; March 1998,  
 Odila Saucedo-Cardenas et al; "Nurrl essential for the  
 induction of the Dopaminergic phenotype and the survival  
 of ventral mesenphalic late dopaminergic  
 Precursor neurons", Page 4013-4018.

D2: Science, Volume 276, April 1997,  
 Rolf Zetterström et al, "Dopamine neuron Agenesis in  
 Nurrl-Deficient mice", Page 248-580.

D3: US 5783680 A.

Document D1, D2 and D3 are considered to constitute the state of the art. Nurrl is a member of the nuclear receptor superfamily of transcription factors that is expressed predominantly in the central nervous system including developing and mature dopaminergic neurons.

The amended claims filed with letter of 2001-09-04 differ from the claims as originally filed in the removal of the terms "functional fragment" or "variant thereof", in claim 1.

The invention according to amended claims 1-16, 18-21, 26-28 and partially 23-25, is thus novel and is considered to involve an inventive step.

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## CLAIMS

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1. An isolated Nurrl gene including one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del).
2. A fragment according to claim 1, which comprises the exons of the Nurrl gene.
3. A fragment according to claim 1 or 2, which comprises exon 3 of the Nurrl gene.
4. A fragment according to any one of claims 1-3, which comprises the mutation Met97Val.
5. A fragment according to any one of claims 1-3, which comprises the mutation His103Arg.
6. A fragment according to any one of claims 1-3, which comprises the mutation Tyr121del or Tyr122del.
7. A nucleic acid capable of specifically hybridising to a gene or a fragment according to any one of claims 1-6.
8. A vector comprising a nucleic acid according to any one of claim 1-7.
9. A recombinant cell comprising a vector according to claim 8.
10. An isolated cell carrying one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del) in its genome.
11. A cell culture comprising cells according to claim 9 or 10, which cells are immortalised cells, such as mammalian cells.
12. A protein or a peptide encoded by a gene or a gene fragment according to any one of claims 1-7.
13. A protein or peptide according to claim 12, which includes a Val residue in the position corresponding to amino acid no. 97 of the wild type Nurrl protein.
14. A protein or peptide according to claim 12, which includes an Arg residue in the position corresponding to amino acid no. 103 of the wild type Nurrl protein.
15. A protein or peptide according to claim 12, which does not include any Tyr residue in the position corresponding to amino acid no. 121 or 122 of the wild type Nurrl protein.

16. A method of screening for pharmaceutically active substances, wherein a nucleotide according to any one of claims 1-7 or a protein or peptide according to any one of claims 12-15 is used as a lead compound to identify substances capable of altering the biological effect of said nucleotide, or protein or peptide.
17. A method for the production of a pharmaceutical composition comprising the method according to claim 16 and furthermore mixing the substance identified with a pharmaceutically acceptable carrier.
18. An antibody raised against a protein or peptide according to any one of claims 12-15.
19. A transgenic, non-human animal, such as a mouse or a rat, comprising a gene or a gene fragment according to any one of claims 1-7.
20. A transgenic mouse comprising a mutation in the chromosome corresponding to the human chromosome 2q22-23 of said mouse, or an ancestor thereof, introduced at an embryonic stage such that said transgene replaces an endogenous allele resulting in said mutation, which transgenic mouse comprises one or more mutations selected from the group consisting of Met97Val (M97V), His103Arg (H103R), Tyr121del (Y121del) and Tyr122del (Y122del).
21. A method of screening for pharmaceutically active substances, wherein an animal according to claim 19 or 20 is used as a lead to identify substances capable of altering the biological effect of a nucleotide according to any one of claims 1-7 and/or a protein or peptide according to any one of claims 12-15.
22. A method for the production of a pharmaceutical composition comprising the method according to claim 21 and furthermore mixing the substance identified with a pharmaceutically acceptable carrier.
23. An antibody according to claim 18 or a substance according to claim 17 or 22 for use as a medicament.
24. Use of an antibody according to claim 18 or a substance according to claim 17 or 22 in the manufacture of a medicament

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for the treatment and/or prevention of psychotic conditions, such as schizophrenia and/or manic depressive disorder.

25. A pharmaceutical preparation comprising an antibody according to claim 18 or a substance according to claim 17 or 22 together with a pharmaceutically acceptable carrier.

26. A method of detecting the presence of a mutation in exon 3 of the Nurrl gene, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del, said method comprising obtaining a biological sample from a mammalian, such as a human, subject and analysing said sample for said mutation.

27. A method according to claim 26, wherein the biological sample is analysed by isolating DNA from said sample, amplifying said DNA, hybridising said DNA to a labeled oligonucleotide probe that specifically hybridises to mutant DNA containing a G as the first base of codon no 97; a G as the second base of codon no 103; or a deleted TAC in codon no 121 or 122, or to the close vicinity of said DNA..

28. A kit for performing the method according to claim 26 or 27, which kit comprises:

- (a) reagents for amplification of one or more of the mutated sites; and/or
- (b) enzymes for specific cleavage of DNA; and
- (c) optionally suitable labels.

29. A method of treating and/or preventing a condition associated with schizophrenia and/or manic depression in a patient in need of therapy, wherein a mutation in exon 3 of the Nurrl gene is corrected, which mutation is selected from the group consisting of Met97Val, His103Arg, Tyr121del and Tyr122del.

30. A method according to claim 29, wherein the DNA of one or more of said mutations is replaced by DNA having the native, non-mutated base sequence using a vector suitable for transfecting the patient.

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31. A method according to claim 29, wherein cells comprising the native, non-mutated base sequence in the positions corresponding to one or more of said mutations are introduced in said patient.